

WHAT IS CLAIMED:

1. A recombinant factor VIII comprising a point mutation in or near at least one calcium binding site of a wild-type factor VIII, wherein the recombinant factor VIII has a specific activity that is higher than that of the wild-type factor VIII.
2. The recombinant factor VIII according to claim 1, wherein the at least one calcium binding site is in the A1 domain.
3. The recombinant factor VIII according to claim 2, wherein the point mutation comprises a substitution of the amino acid residue at position 113 of SEQ ID NO:2.
4. The recombinant factor VIII according to claim 3, wherein the substitution at residue 113 of SEQ ID NO:2 is selected from the group consisting of E113A, E113V, E113I, E113N, E113L, E113G, and E113M.
5. The recombinant factor VIII according to claim 3, wherein the substitution at residue 113 of SEQ ID NO:2 is E113A.
6. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII has a specific activity at least about twice as great as the activity of the wild-type factor VIII.
7. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII has a higher binding affinity for Ca^{2+} compared to that of the wild-type factor VIII.
8. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII has a higher binding affinity for Mn^{2+} compared to that of the wild-type factor VIII.
9. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII consists of domains A1, A2, A3, C1, and C2, or portions thereof.

10. The recombinant factor VIII according to claim 9 wherein domains A1 and A2 are present on a heavy chain and domains A3, C1, and C2 are present on a light chain.

11. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII comprises one or more domains, or portions thereof, from human factor VIII and one or more domains, or portions thereof, from a non-human mammalian factor VIII.

12. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII has a circulating half-life value that is equivalent to or greater than that of the wild-type factor VIII.

13. The recombinant factor VIII according to claim 1, wherein the recombinant factor VIII is substantially pure.

14. The recombinant factor VIII according to claim 1 wherein the recombinant factor VIII further comprises modified inactivation cleavage sites.

15. The recombinant factor VIII according to claim 1 wherein the recombinant factor VIII further comprises factor IXa and/or factor X binding domains modified to enhance the affinity of the recombinant factor VIII for one or both of factor IXa and factor X.

16. The recombinant factor VIII according to claim 1 wherein the recombinant factor VIII further comprises modified sites that enhance secretion in culture.

17. The recombinant factor VIII according to claim 1 wherein the recombinant factor VIII further comprises modified serum protein binding sites that enhance the circulating half-life thereof.

18. The recombinant factor VIII according to claim 1 wherein the recombinant factor VIII further comprises at least one glycosylation recognition sequence that is effective in decreasing antigenicity and/or immunogenicity thereof.

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19. A pharmaceutical composition comprising the recombinant factor VIII according to claim 1.

20. The pharmaceutical composition according to claim 19 further comprising a stabilizer.

21. The pharmaceutical composition according to claim 19 further comprising a delivery vehicle.

22. The pharmaceutical composition according to claim 19 further comprising a pharmaceutically acceptable carrier.

23. An isolated nucleic acid molecule encoding a recombinant factor VIII according to claim 1.

24. The isolated nucleic acid molecule according to claim 23, wherein the nucleic acid encodes a substitution of the amino acid at position 113 of SEQ ID NO:2.

25. The isolated nucleic acid molecule according to claim 24, wherein the substitution at residue 113 of SEQ ID NO:2 is selected from the group consisting of E113A, E113V, E113I, E113N, E113L, E113G, and E113M.

26. The isolated nucleic acid molecule according to claim 24, wherein the substitution at residue 113 of SEQ ID NO:2 is E113A.

27. The isolated nucleic acid molecule according to claim 23, wherein the recombinant factor VIII further comprises modified inactivation cleavage sites.

28. The isolated nucleic acid molecule according to claim 23, wherein the recombinant factor VIII further comprises factor IXa and/or factor X binding domains modified to enhance the affinity of the recombinant factor VIII for one or both of factor IXa and factor X.

29. The isolated nucleic acid molecule according to claim 23, wherein the recombinant factor VIII further comprises modified sites that enhance secretion in culture.

30. The isolated nucleic acid molecule according to claim 23, wherein the recombinant factor VIII further comprises modified serum protein binding sites that enhance the circulating half-life thereof.

31. The isolated nucleic acid molecule according to claim 23, wherein the recombinant factor VIII further comprises at least one glycosylation recognition sequence that is effective in decreasing antigenicity and/or immunogenicity thereof.

32. The isolated nucleic acid molecule according to claim 23, wherein the nucleic acid is RNA.

33. The isolated nucleic acid molecule according to claim 23, wherein the nucleic acid is DNA.

34. The isolated nucleic acid molecule according to claim 33, wherein the isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO: 1, as modified at codon 113 (nt 337-339).

35. A recombinant DNA expression system comprising a DNA molecule according to claim 33.

36. The recombinant DNA expression system according to claim 35, wherein the DNA molecule is in sense orientation relative to a promoter.

37. A host cell comprising a nucleic acid molecule according to claim 23.

38. A host cell comprising the DNA molecule according to claim 33.

39. The host cell according to claim 38, wherein the DNA molecule is in an expression system.

40. The host cell according to claim 38, wherein the host cell is an animal cell, a bacterial cell, an insect cell, a fungal cell, a yeast cell, a plant cell, or an algal cell.

41. A method of making a recombinant factor VIII comprising:
growing a host cell according to claim 36 under conditions
whereby the host cell expresses the recombinant factor VIII; and
isolating the recombinant factor VIII.
42. The method according to claim 41, wherein said growing is carried out *in vitro* in a growth medium.
43. The method according to claim 42, wherein the growth medium comprises von Willebrand Factor.
44. The method according to claim 41, wherein the host cell comprises a transgene encoding von Willebrand Factor.
45. The method according to claim 42, wherein the recombinant factor VIII is secreted into the growth medium, said isolating comprising isolating the recombinant factor VIII from the growth medium.
46. The method according to claim 41 further comprising:
disrupting the host cell prior to said isolating, wherein said isolating comprises isolating the recombinant factor VIII from cellular debris.
47. A method of making a recombinant factor VIII having increased specific activity compared to that of a wild-type factor VIII, the method comprising:
altering the amino acid sequence of wild-type factor VIII to yield a recombinant factor VIII, wherein said altering comprises introducing at least one point mutation in or near at least one calcium binding site of the wild-type factor VIII; and
determining whether the recombinant factor VIII has increased specific activity compared to that of the wild-type factor VIII.
48. A method of treating an animal for hemophilia A, the method comprising:
administering to an animal exhibiting hemophilia A an effective amount of the recombinant factor VIII according to claim 1, whereby the animal exhibits effective blood clotting following vascular injury.

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49. The method according to claim 48, wherein the effective amount comprises between about 10 to about 50 units/kg body weight of the animal.

50. The method according to claim 48 wherein the animal is a mammal.

51. The method according to claim 50 wherein the mammal is selected from the group consisting of human, rat, mouse, guinea pig, dog, cat, monkey, chimpanzee, orangutan, cow, horse, sheep, pig, goat, rabbit, and chicken.

52. The method according to claim 48 further comprising:
periodically repeating said administering.